Exhibit 1

J Clin Invest. 2008 Feb;118(2):583-96.

Role of Gas6 in erythropoiesis and anemia in mice.

Angelillo-Scherrer A, Burnier L, Lambrechts D, Fish RJ, Tjwa M, Plaisance S, Sugamele R, DeMol M, Martinez-Soria E, Maxwell PH, Lemke G, Goff SP, Matsushima GK, Earp HS, Chanson M, Collen D, Izui S, Schapira M, Conway EM, Carmeliet P.

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Many patients with anemia fail to respond to treatment with erythropoietin (Epo), a commonly used hormone that stimulates erythroid progenitor production and maturation by human BM or by murine spleen. The protein product of growth arrest-specific gene 6 (Gas6) is important for cell survival across several cell types, but its precise physiological role remains largely enigmatic. Here, we report that murine erythroblasts released Gas6 in response to Epo and that Gas6 enhanced Epo receptor signaling by activating the serine-threonine kinase Akt in these cells. In the absence of Gas6, erythroid progenitors and erythroblasts were hyporesponsive to the survival activity of Epo and failed to restore hematocrit levels in response to anemia. In addition, Gas6 may influence erythropoiesis via paracrine erythroblast-independent mechanisms involving macrophages. When mice with acute anemia were treated with Gas6, the protein normalized hematocrit levels without causing undesired erythrocytosis. In a transgenic mouse model of chronic anemia caused by insufficient Epo production, Gas6 synergized with Epo in restoring hematocrit levels. These findings may have implications for the treatment of patients with anemia who fail to adequately respond to Epo.

Publication Types:

- Research Support, Non-U.S. Gov't

PMID: 18188450 [PubMed - indexed for MEDLINE]

PMCID: PMC2176185

Exhibit 2

1: Respir Physiol Neurobiol. 2006 Aug;153(1):14-22. Epub 2005 Dec 5.

Time course of ventilatory acclimatisation to hypoxia in a model of anemic transgenic mice.

Macarlupú JL, Buvry A, Morel OE, León-Velarde F, Richalet JP, Favret F.

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We questioned the assumption that polycythemia is essential for adaptation to chronic hypoxia. Thus, the objective of our study was to determine if anemic Epo-TAg(h) mice could survive in hypoxia despite low oxygen carrying capacity. We explored the possibility that ventilatory acclimatisation is involved in the strategy used by anemic transgenic mice to adapt to chronic hypoxia. Epo-TAg(h) and Wild Type mice were exposed during 2 weeks at a barometric pressure of 450 Torr. After 1, 5 and 14 days of exposure, ventilation at different inspired oxygen fraction was measured in both groups. Ventilation during acclimatisation to hypoxia was significantly greater in Epo-TAg (h) than in Wild Type. The difference was mainly due to a higher tidal volume that could explain a higher arterial PO2 in Epo-TAg(h) mice. Epo-Tag(h) mice did not develop right ventricle hypertrophy after 2 weeks of exposure to hypoxia while Wild Type did. Hemoglobin concentration was 60% lower in anemic mice versus Wild Type after acclimatisation. In conclusion, ventilatory acclimatisation contributed to the adaptation of Epo-Tag(h) mice in chronic hypoxia despite low arterial oxygen carrying capacity.

Publication Types:
- Comparative Study

PMID: 16330260 [PubMed - indexed for MEDLINE]

Exhibit 3

Respir Physiol Neurobiol. 2006 Jan 25;150(1):19-26.

Characterisation of the ventilatory response to hypoxia in a model of transgenic anemic mice.

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Both polycythemia and the increase in hypoxic ventilatory response (HVR) are considered as important factors of acclimatization to hypoxia. The objective of this study was to characterise the ventilation pattern at different inspired oxygen fraction in a model of chronic anemic mice. These mice have a targeted disruption in the 5' untranslated region of the Epo gene that reduces Epo expression such that the homozygous animal is severely anemic. Ventilation in normoxia in Epo-TAg(h) mice was significantly greater than in wild type, and the difference was mainly due to a higher tidal volume. HVR was higher in Epo-TAg(h) mice at every FIO2 suggesting a higher chemosensitivity. Resting oxygen consumption was maintained in anemic mice. Maximal oxygen consumption was 30% lower while hemoglobin was 60% lower in anemic mice compared to wild type. This small decrease in maximal oxygen consumption is probably due a greater cardiac output and/or a better tissue oxygen extraction and would allow these anemic mice to acclimatize to hypoxia in spite of low oxygen carrying capacity. In conclusion, Epo-TAg(h) anemic mice showed increased ventilation and hypoxic ventilatory response. However, whether these adaptations will contribute to acclimatization in chronic hypoxia remains to be determined.

Publication Types:
- Comparative Study

PMID: 15878311 [PubMed - indexed for MEDLINE]

Exhibit 4

Blood. 2002 Oct 1;100(7):2406-13.

Long-term reversal of chronic anemia using a hypoxia-regulated erythropoietin gene therapy.

Binley K, Askham Z, Iqball S, Spearman H, Martin L, de Alwis M, Thrasher AJ, Ali RR, Maxwell PH, Kingsman S, Naylor S.

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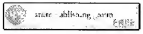
Anemia is a common clinical problem, and there is much interest in its role in promoting left ventricular hypertrophy through increasing cardiac workload. Normally, red blood cell production is adjusted through the regulation of erythropoietin (Epo) production by the kidney. One important cause of anemia is relative deficiency of Epo, which occurs in most types of renal disease. Clinically, this can be corrected by supplementation with recombinant Epo. Here we describe an oxygen-regulated gene therapy approach to treating homozygous erythropoietin-SV40 T antigen (Epo-TAg(h)) mice with relative erythropoietin deficiency. We used vectors in which murine Epo expression was directed by an Oxford Biomedica hypoxia response element (OBHRE) or a constitutive cytomegalovirus (CMV) promoter. Both corrected anemia, but CMV-Epo-treated mice acquired fatal polycythemia. In contrast, OBHRE-Epo corrected the hematocrit level in anemic mice to a normal physiologic level that stabilized without resulting in polycythemia. Importantly, the OBHRE-Epo vector had no significant effect on the hematocrit of control mice. Homozygous Epo-TAg(h) mice display cardiac hypertrophy, a common adaptive response in patients with chronic anemia. In the OBHRE-Epo-treated Epo-TAg(h) mice, we observed a significant reversal of cardiac hypertrophy. We conclude that the OBHRE promoter gives rise to physiologically regulated Epo secretion such that the hematocrit level is corrected to healthy in anemic Epo-TAg(h) mice. This establishes that a hypoxia regulatory mechanism similar to the natural mechanism can be achieved, and it makes EPO gene therapy more attractive and safer in clinical settings. We envisage that this control system will allow regulated delivery of therapeutic gene products in other ischemic settings.

PMID: 12239150 [PubMed - indexed for MEDLINE]

Kidney Int. 2002 Oct;62(4):1395-401.



Delivery of erythropoietin by encapsulated myoblasts in a genetic model of severe anemia.

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BACKGROUND: Existing animal models of anemia inadequately reflect the hematocrit usually present in chronic renal failure (CRF) patients and do not permit long-term treatment studies. The transgenic mouse strain 134.3LC (Epo-TAg(H)) displays a severe chronic anemia resembling that observed clinically during CRF, while displaying an active, normal life span. This phenotype makes it a particularly interesting mouse model for testing erythropoietin (Epo)-based gene transfer strategies. METHODS: Ex vivo gene therapy was employed to administer mouse Epo to homozygous anemic Epo-TAg(H) mice. Encapsulated C(2)C(12) myoblasts genetically engineered to secrete 163 IU mouse Epo/10(6) cells/day were subcutaneously transplanted on the dorsal flank of the mice. Efficacy of delivered Epo was monitored by weekly measurements of animal hematocrit. RESULTS: Most treated homozygous Epo-TAg(H) mice displayed only a transient rise in hematocrit before eventually decreasing to levels as low as 3%. Administering the immunosuppressor anti-CD4+ monoclonal antibody (mAb) to homozygous Epo-TAg(H) mice, beginning at the time of implantation, permitted a rise in hematocrit that remained stable at elevated levels in cases of continued immunosuppression. CONCLUSIONS: Mice having the T antigen insertion in both Epo alleles appeared to develop an immune response to the natural mouse Epo delivered by encapsulated cells. By preventing this reaction using immunosuppression, we demonstrate that encapsulated myoblasts can deliver therapeutic doses of mouse Epo systemically and restore hemopoiesis in a genetic model of severe anemia.

PMID: 12234311 [PubMed - indexed for MEDLINE]